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(54) MEASUREMENT OF ENZYME ACTIVITY

(57)Abstract:
PROBLEM TO BE SOLVED: To simply determine an enzyme activity with high sensitivity by using polypeptide as a substrate, a linking a fluorescent pigment to the substrate, separating the reaction solution through a fluorescence-detecting isoelectric focusing method, then detecting the fluorescent light.
SOLUTION: In the measurement of activity of an enzyme such as protease using a polypeptide as a substrate, this substrate is trypsin or chymotrypsin and bonded through a binding group to a pigment bearing a fluorescent group, for example, rhodamine, fluoresceine, cyanine, indocyanine, indocarbocyanine, pyronine, lucifer yellow, quinacrine, squaric acid, coumarin, fluoroanthenilmaleimide, anthracene and the like. The substrate is added to a buffer solution containing an enzyme sample, then an enzyme inhibitor is added to stop the reaction and the reaction product is separated through a fluorescence-detecting isoelectric focusing method. The separated fluorescent substance is excited to detect the fluorescent emission whereby the objective enzyme activity is determined in high sensitivity and accuracy.

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